The effect of the androgens on lymphocyte infiltration into the porcine endometrium

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I. Abstract

Litter size is an important aspect of the swine industry and sows are often genetically selected for large litter sizes because of the economic importance of such a trait. Finding alternate ways to influence the number of pigs born, in addition to genetic manipulation could be of economic value to producers**.** Androgens, including testosterone and dihydrotestosterone (DHT) are used to increase a sow's ovulation rate to maximize litter size. However, these hormones can have a negative effect on the health of the sow's uterus. When DHT was used in high dosages, the uterine contents were opaque and infected which suggests that the hormone negatively influences the lymphocyte infiltration during proestrus (Cardenas et al. 2002).

When pigs mate, the uterine environment is exposed to a large amount of foreign material that can potentially cause serious infections and limit the uterus' ability to support growing embryos. Because of the volume of the ejaculate and the length of the uterine horns, swine rely on the lymphocyte infiltration that occurs during the proestrus period to fight off foreign material and bacteria that can compromise uterine health.

In order to examine the effect that androgens have on the lymphocyte infiltration into the porcine endometrium, twelve gilts were divided into six groups and treated with either testosterone, dihydrotestosterone, testosterone plus flutamide, DHT plus flutamide, flutamide or oil from day 13 to day 18 of the estrus cycle. Then the number of lymphocytes in a section of each uterine horn was quantified.

The concentration of lymphocytes that were observed in the endometrium of the gilts treated with DHT was significantly lower than in the control group. This is consistent

with the occurrence of uterine infections and may explain the prevalence of infection in gilts that are treated with DHT.

II. Introduction

Swine have a long, five day period of proestrus, to allow lymphocyte infiltration into the wall of the uterus to fight the microflora that are introduced into the reproductive tract when mating occurs. Normal proestrus is characterized by increased blood flow to the uterus, increased edema, and increased lymphocyte infiltration. Boars ejaculate into the anterior portion of the cervix or into the uterus about 200 to 500 ml. Androgen treatments might compromise the uterus's ability to fight off bacteria, causing infections which may be due to changes in the lymphocyte concentration in the uterus.

Exogenous testosterone was initially of interest because it increases ovulation rate but at higher dosages it also inhibits embryonic survival and uterine function (Cardenas 1997). Testosterone is an androgen that has been shown in several experiments at low dosages to increase ovulation rate and the number of concepti which could influence litter size in swine. Testosterone is an intermediate in the synthesis of estrogen which could be a method in which exogenous testosterone affects ovulation.

Dihydrotestosterone is another androgen that also has an effect on ovulation rate. DHT is an androgen that is not aromatizable into estrogens. DHT acts through the androgen receptor to increase FSH receptors and FSH secretion, which could both help in the maturation process of follicles (Cardenas et al. 2002). Previous research demonstrated that DHT increased ovulation but hurt embryo survival to day 11 of

gestation. The uterine contents after DHT treatment during the period of follicular growth were found to be opaque and infected (Cardenas 2002).

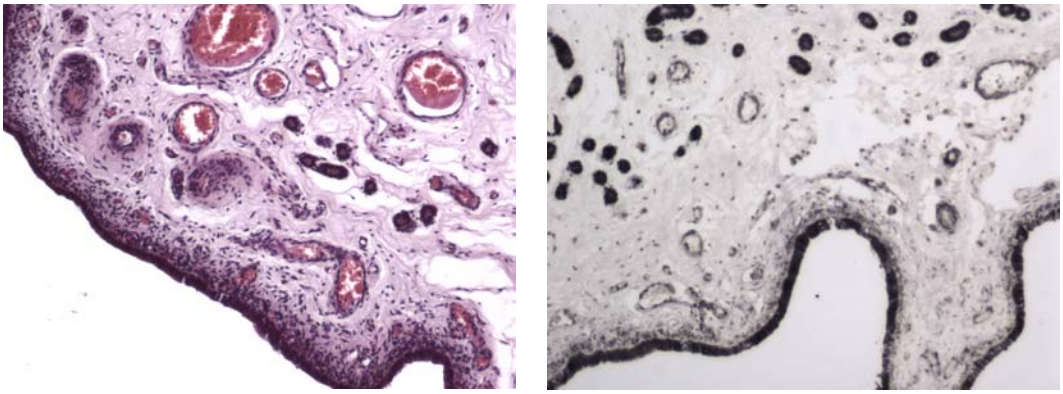
Since there is evidence DHT is linked to the occurrence of uterine infections, it is possible that the hormone may have an effect on the concentration of lymphocytes that infiltrate the uterus during the estrous cycle.

Previous research concerning the use of androgens to increase a sow's fertility did not address the changes in the lymphocyte population in the uterus. This could have important ramifications since uterine health influences reproduction. The goal for this proposed research was to qualify the changes that occur in the uterus's lymphocyte concentration that may hinder the ability of the uterus to fight infections. It was hypothesized that treating gilts with DHT would decrease the number of lymphocytes that infiltrate the porcine uterine endometrium during the period of proestrus. The other treatments were not expected to affect the lymphocyte infiltration.

III. Materials and Methods

Twelve gilts were divided into 6 treatment groups (n=2 in each group). Each gilt was injected intramuscularly with testosterone, DHT, flutamide, flutamide and testosterone, flutamide and DHT, or oil (vehicle) starting on day 13 of their estrus cycle and continuing until day 18. Flutamide was used because it is an antagonist to the androgen receptor. It prohibits the action of DHT on the androgen receptor which would limit the effects that DHT has on ovulation rate and it was hypothesized that it would also limit the potential effect on the lymphocyte infiltration.

On day 19, ovariohysterectomies were surgically performed on the gilts and a small section of each uterine horn was collected for analysis. The sections of uterus were fixed with 4% paraformaldehyde and embedded in paraffin. Immunohistochemical analysis was performed using a rabbit polyclonal antibody to CD2 (ab37211 from Abcam, Cambridge, MA). The antibody was specific for the surface antigen of the human T cell lineage, CD2, which is expressed on peripheral blood T cells. It is one of the earliest T cell markers, being present on more than 95% of thymocytes. It is also found on some natural killer cells but not on B lymphocytes. The stained lymphocytes were verified as lymphocytes using a Hematoxylin and Eosin stained section of uterine endometrium (Fig 1a). A light microscope, fitted with a digital camera, was used at 100x magnification to take pictures of the endometrium. A photo was taken of two separate sections of each slide and the number of lymphocytes on each photo was counted and averaged across gilts in each group (Fig. 1b).



A B

Figure 1: Staining that was performed to quantify lymphocyte infiltration of the endometrium. Hematoxylin and Eosin stained endometrium (A). Endometrium stained with rabbit polyclonal antibody to CD2 (B).

IV. Results

The observations that were made in this experiment were not expected. Treating gilts with DHT alone decreased the number of lymphocytes that infiltrated the endometrium at day 19 of the estrus cycle compared to the control group of gilts which was the hypothesized result. However, combining the DHT with flutamide was not significantly different than treating with DHT alone which was not expected (Fig. 2).

The testosterone treatment did not affect the number of lymphocytes in the endometrium compared to the control and the testosterone plus flutamide treatment produced similar results (Fig. 2).

Treatment with flutamide decreased the number of lymphocytes that infiltrated the endometrium compared to the control group of animals which was not an expected result (Fig. 2).

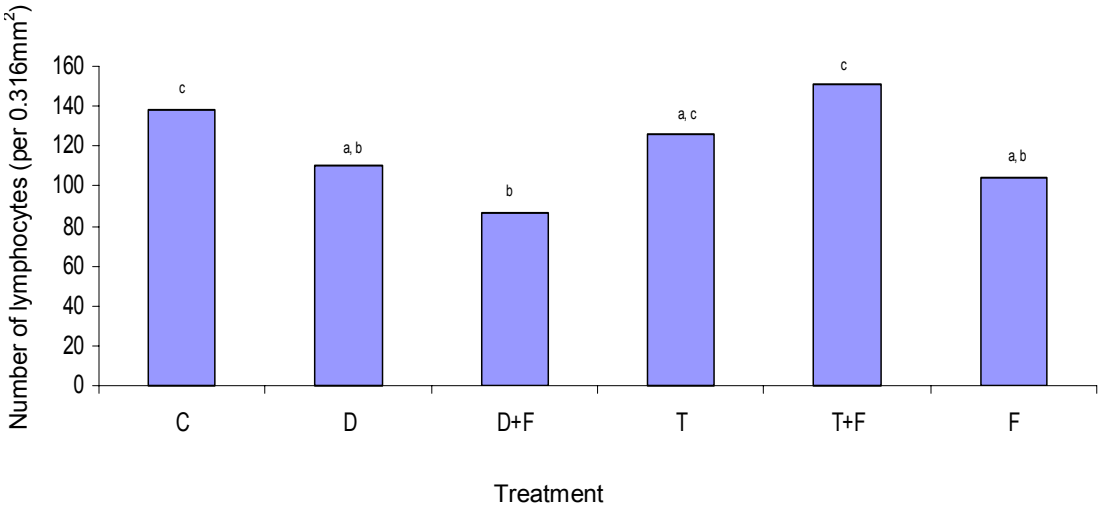


FIGURE 2: Lymphocyte infiltration into the endometrium in animals treated with oil (C), DHT (D), DHT+flutamide (DF), Testosterone (T), Testosterone+Flutamide (TF), or Flutamide (F) from day 13 of the estrus cycle to day 18.

a,b,c Means lacking a common superscript differ ($P<0.05$)

V. Discussion

The mechanism by which androgens function to increase the ovulation rate and litter size of pigs is unclear but it appears that the stronger androgen, DHT increases FSH secretion from the pituitary and increases receptors for FSH in granulosa cells of the preovulatory follicle compared to the weaker androgen, testosterone. Unfortunately, there are limits to improving fertility with androgens because at some dosage point, androgens negatively affect uterine function.

It was hypothesized that the gilts treated with DHT alone would have fewer lymphocytes that infiltrated the endometrium than all other treatments. We expected that number of lymphocytes found in the endometrium of the pigs in the five other groups

would not differ from each other. The testosterone treatment in previous research did not cause uterine infections like the DHT treatment did (Cardenas 1997). The flutamide was added to interfere with the action of the DHT so that the DHT would not affect the lymphocyte infiltration. Therefore the testosterone treated gilts would have the same number of lymphocytes as the control group. The expected results of the flutamide treatment and the DHT plus flutamide treatment were that the number of lymphocytes that infiltrated the endometrium would be the same as the control group. The flutamide would block the mode of action of DHT making it ineffective in increasing ovulation and would also prevent the uterine infections. The flutamide was not expected to have any effect on the lymphocyte infiltration.

Fewer lymphocytes were counted in the endometrium of the gilts that were treated with DHT. These observations are consistent with, and perhaps explain the mechanism of uterine infections.

Treatment with testosterone did not alter lymphocyte infiltration relative to the vehicle treated gilts. Testosterone can either be converted to estrogen or bind to the androgen receptor. This observation was expected because testosterone is a weaker agonist for the androgen receptor than DHT which may explain the lack of an effect on uterine function relative to DHT.

Treatment of gilts with testosterone plus flutamide was not different from testosterone alone because the testosterone did not affect the uterine health as observed (Cardenas 1997). The flutamide in this case did not effect the lymphocyte infiltration either, as expected.

Treatment of gilts with DHT plus flutamide was not different from treatment with DHT alone. We expected the two treatments to differ because the flutamide would block the androgen receptor and make the lymphocyte infiltration similar to the control group of animals. However, the results that were observed were different from what was expected. This could have been because of the small number of gilts that were observed in each treatment group.

Surprisingly, the gilts treated with flutamide also had fewer lymphocytes in their endometrium compared to the control animals. This observation was not expected but may be explained by a low number of observations (n=2), a toxic effect of flutamide to lymphocyte function or infiltration, or the treatment interfered with the ability to count lymphocytes.

VI. References

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Cárdenas H, Herricks, J. R., and Pope WF. 2002. Increased ovulation rate in gilts treated with dihydrotestosterone. Reproduction 123 527-533 .

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